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Analysis of polychlorinated biphenyls in shellfish collected from a market

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Abstract : We collected seven species of shellfish; all originating from the southern coastal areas of Korea, from a market every three months from Dec. 2001 to Sept. 2002, and determined the total polychlorinated biphenyl (PCB) levels by the sum of 26 individual congener levels. A GC-ECD system was applied for identification and quantification of these PCB congeners. Mussel showed the highest level in Sept. 2002 at 34.5 ng/g dry weight (d.w.). All species except mussel showed the lowest total PCB level in Dec. 2001 and their levels in tissue ranged from 0.6 to 5.5 ng/g d.w. The total PCB levels ranged from 0.8 to 17.3 ng/g in Mar. 2002, 2.2 to 9.5 ng/g in June 2002, and 1.8 to 34.5 ng/g d.w. in Sept. 2002. The principal congener group was penta-CBs, which accounted for 32% of the total PCBs, followed by hexa-CBs at 23%, and tetra-CBs at 21%.

Key words : polychlorinated biphenyls, shellfish, pcbs, oyster, mussel

1. Introduction

The southern coastal areas are the prevailing habitats of many species of shellfish in Korea, covering a linear distance of some 300 km including estuaries of four rivers (the Nakdong, Seomjin, Bosung, and Tamjin), several bays (bays Jinhae, Masan, Jinju, Gwangyang, Yeosu, Soonchun, and Bosung), and a lot of small islands. Coastal and estuarine areas may incur a higher exposure risk to biota from various pollutants than the deep sea. Although the production and practical use of PCBs was banned more than 20 years ago in Korea, they are still ubiquitous in every environmental media. It has been reported that contaminant levels in some marine organisms can reflect levels in their environment.[1-3] Shellfish might be better bioindicators of marine pollution than other marine organisms due to their more limited habitat area. Bivalves such as mussels have been used as biomonitors to evaluate the bioavailability and effects of contaminants present in estuary and coastal zones.[4-8] Polychlorinated biphenyls have been of great concern because of their highly bioaccumulative nature and toxic biological effects.[9-13] The PCB levels in the environment have gradually been reduced since the 1970s following restrictions on PCB use.[14,15]

Short-necked clam-1 (*Cyclina sinensis*), granular ark (*Tegillarca granosa*), mussel (*Mytilus coruscus*), oyster (*Crassostrea gigas*), short-necked clam-2 (*Meretrix lamarckii*), Japanese littleneck (*Tapes philippinarum*), and purplish Washington clam (*Saxidomus purpuratus*) are common in coastal water of Korea.

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In the present study, we reported the total PCB levels and homolog profiles of PCBs in the seven species of shellfish originating from the southern coastal areas of Korea.

2. Materials and Methods

2.1. Sample collection

About 1 kg of each shellfish was collected from a market every three months from Dec. 2001 to Sept. 2002. The collected shellfish were estimated at 2-3 years old and the size was as follows: clam-1, 9-10 cm; granular ark, 5-6 cm; mussel, 7-8 cm; oyster, 9-10 cm; clam-2, 5-6 cm; Japanese littleneck, 5-6 cm; and Washington clam, 8-9 cm. After removing the shell, 400 g of each sample was homogenized using a homogenizer (Ika Co. Germany) and freeze dried for more than 24 hours at -60°C, and then stored in a refrigerator after being sealed in bottles as fine powder.

2.2. Pretreatment of samples

The yield recovery rates were obtained from the certified reference material CARP-1 (National Research Council of Canada), through triplicate determinations. The analyzed PCB congeners in CARP-1 were PCB 52, 101, 105, 118, 138, 153, 170, 180, and 187, and their recovery rates were 84.7%, 124%, 101%, 97.0%, 126%, 89.9%, 102%, 99.4%, and 112% respectively. The recovery rates were acceptable, so the experimental results were not corrected. The lipid contents were measured in accordance with the US EPA Method 1668A. Florisil (J.T. Baker, 60-100 Mesh) and silica gel (Wakogel S-1, Wako Pure Chemical Industries, Ltd.) were activated for 9 hours at 130°C, and acid silica gel was prepared by applying silica gel/conc. H_2SO_4 (100 g : 24 mL), followed by homogenization. A Florisil and silica gel chromatography column was prepared by packing a column, from bottom to top, with anhydrous sodium sulfate, acid silica gel, anhydrous sodium sulfate, Florisil, and anhydrous sodium sulfate.

High-purity acetone (Merck, pesticide residue analysis grade) and n-hexane (Merck, organic trace analysis grade) were used as the solvents for the extraction and cleanup processes. Anhydrous sodium sulfate (Katayama, pesticide residue analysis grade) was dried for 4 hours at 130°C and used to remove trace levels of water in the extracts.

Freeze-dried samples (5 g) of individual shellfish soft tissues were soxhlet extracted for 16 hours using 300 mL of 1 : 1 (v/v) acetone : n-hexane mixed solvent. The extract was passed through anhydrous sodium sulfate and concentrated to a few milliliters using a rotary evaporator, and the volume of the extract was further reduced to about 1 mL under a gentle stream of ultra-pure nitrogen gas. The extract was then eluted slowly with 120 mL of n-hexane by passing it through a Florisil-acid silica gel column. The eluent was concentrated to a few milliliters using a rotary evaporator and the final volume was further reduced to 1.0 mL under a gentle stream of ultra-pure nitrogen gas.

2.3. Analysis

The method detection limit (MDL) of individual PCB congeners (Dr. Ehrenstorfer GmbH) was determined by analyzing five blank samples. The standard deviation (stdv) of the measured value for each congener was obtained, and then the detection limit was calculated with the equation.[16]

MDL = 3 stdv.

The MDLs for the congeners ranged from <0.01 to 0.07 ng/g on a dry weight basis. depending on the degree of chlorination. The seven point (0.1, 0.5, 1, 5, 10, 50, and 100 ng/mL) calibration curves were constructed from the ratio of the peak area obtained with a GC-ECD (^{63}Ni electron capture detector) system from 1 μl of standard solution of individual PCB congeners. The total PCB levels by the sum of 26 individual congeners were determined, including eighteen congeners (PCB 8, 18, 28, 44, 52, 66, 77/110, 101, 105, 118, 126, 128, 138, 153, 169, 170, 180, and 187) recommended by the NOAA[17] (National Oceanic and Atmospheric Administration) and the United States EPA (Environmental Protection Agency) for the determination of total PCB

levels, as well as eight other coplanar PCB congeners (PCB 114, 123, 156, 157, 158, 166, 167, and 189). A PCB mixture (Dr. Erenstorfer GmbH) containing known levels of 26 congeners was purchased commercially as the PCB standard.

An analysis was performed using an Agilent-6890 gas chromatograph equipped with an electron capture detector and a capillary column (HP-5MS, 5% phenyl methyl siloxane, 30-m length, 0.25-mm inner diameter, and 0.25-μm film thickness). The injector and detector temperatures were maintained at 250°C and 270°C, respectively. The oven temperature was programmed as follows: 70°C for 2 min, increasing at 30°C/min to 170°C, at 5°C/min to 300°C, and then hold for 10 min. The carrier gas was helium, and the flow rate was 1 mL/min, with nitrogen as the makeup gas.

3. Results and Discussion

3.1. Total PCB levels and homolog profiles

Average PCB levels in the blanks ranged from 0.0057 to 0.34 ng/g d.w., and PCB levels in samples were corrected with the blank values. The total PCB levels in shellfish are presented in *Table* 1, and all numerical values averaged from triplicate determinations are reported on a dry weight (d.w.) basis. The moisture content of each shellfish ranged from 72.6% (oyster, Mar. 2002) to 87.2% (clam-2, Sept. 2002) as shown in *Table* 2. The total PCB levels ranged from 0.5 (clam-2, Dec. 2001) to 34.5 (mussel, Sept. 2002) ng/g d.w. The four species, clam-1, granular ark, mussel, and oyster, contained relatively higher levels. The other three species contained much lower levels. PCB levels did not vary significantly through sampling time. There were three to eight-fold variation in PCB levels within each species excluding granular ark, which showed twenty-fold variation. The measured total PCBs in the seven species of shellfish were less than the U.S. FDA (Food and Drug Administration) tolerance limit of 2 g/g on a wet weight basis for fish and shellfish.[18]

Table 1. Total PCB levels in shellfish (ng/g dry weight)

	Dec. 2001	Mar. 2002	June 2002	Sept. 2002
Clam-1	2.1	17.3	9.4	4.2
Granular ark	0.8	16.3	8.9	6.0
Mussel	5.9	6.9	4.5	34.5
Oyster*	5.6	6.0	(no data)	6.4
Clam-2	0.5	4.0	3.3	2.4
Japanese littleneck	0.8	1.2	2.3	2.1
Washington clam	0.8	0.8	2.2	1.6

*: Oyster was not available in June 2002 from a market.

The homolog profile is illustrated in *Fig.* 1. It shows a clear trend of the strong predominance of

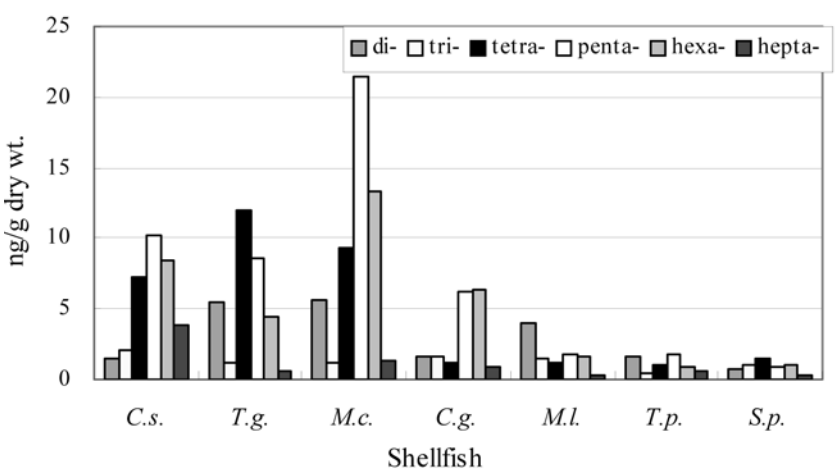

Fig. 1. The homolog profile of each shellfish (*C.s.*: clam-1; *T.g.*: granular ark; *M.c.*: mussel; *C.g.*: oyster; *M.l.*: clam-2; *T.p.*: Japanese littleneck; *S.p.*: Washington clam).

tetra-, penta- and hexa-CBs for relatively highly contaminated species. Another peculiar pattern is the great contribution of di-CBs to the contamination of granular ark, mussel, and clam-2.

Previous study[19] reported total PCB levels in blue mussel from three locations along the southern coastal area, Yosu, Masan, and Busan. Concentrations were in the range rang from 5.8 to 73.3 ng/g wet weight. Another study[20] reported total PCB levels in oyster and mussel originating from several southern coastal areas, ranging from 7.3 ng/g d.w. at Samchunpo to 84.1 ng/g d.w. at Masan Bay. Excluding Masan Bay and Busan Harbor, total PCBs ranged from 7.3 to 26.5 ng/g dry weight. Total PCB levels in mussel and oyster measured from this study were comparable to those obtained from the previous studies performed in Korea excluding Masan Bay and Busan Harbor.

Sericano *et al.*[21] reported the results of International Mussel Watch, which initiated a monitoring programme in Central and South America, including Mexico and the Caribbean, in 1991-1992. The PCB levels in oysters, mussels and other bivalves ranged from 10 to 3,800 ng/g d.w., showing very wide distribution of total PCBs by species and locations. Another mussel (*Mytilus galloprovincialis*) survey,[6] conducted in 1988-1989 at eleven locations

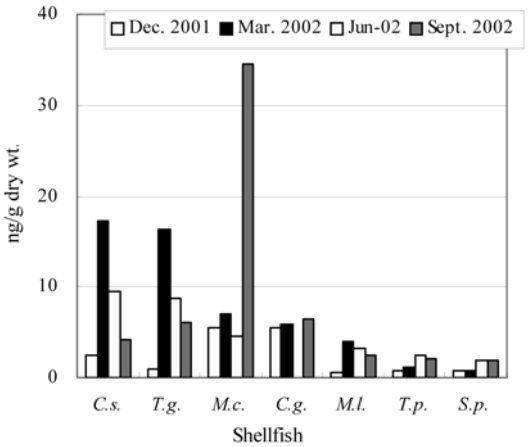

Fig. 2. Total PCB levels of each shellfish (*C.s.*: clam-1; *T.g.*: granular ark; *M.c.*: mussel; *C.g.*: oyster; *M.l.*: clam-2; *T.p.*: Japanese littleneck; *S.p.*: Washington clam).

along the Mediterranean coast of France and Italy, also identified wide distributions of total PCB levels by location, ranging from 50 to 3,500 ng/g d.w. with an average of 526 ± 1,021 ng/g.

Khim *et al.*[19] introduced total PCB levels in blue mussel collected worldwide: Kattegat, Denmark (1985), 3.00-328 ng/g wet weight; northwest Spain (1990-1991), ND-620; Perth, western Australia (1991), <10.0; coastal water of USA (1992 and 1993), 12.0 and 16.0, respectively; and south west Baltic Sea (1990-1991), 4.70-98.5. They also introduced

Table 2. Lipid and moisture content of each shellfish

Shellfish species	Lipid (moisture) content (%)			
	Dec. 2001	Mar. 2002	June 2002	Sept. 2002
Clam-1	4.4 ± 0.4 (83.0)	10.0 ± 1.5 (83.4)	7.0 ± 2.4 (83.3)	2.6 ± 0.7 (82.1)
Granular ark	8.0 ± 0.4 (78.0)	10.0 ± 3.0 (75.7)	7.8 ± 0.6 (81.9)	2.2 ± 0.7 (82.6)
Mussel	8.2 ± 0.4 (80.8)	6.8 ± 0.4 (80.6)	7.2 ± 1.0 (79.9)	11.0 ± 0.9 (82.6)
Oyster	11.0 ± 1.7 (78.2)	5.2 ± 1.4 (72.6)	(no data)	7.4 ± 0.4 (84.2)
Clam-2	3.3 ± 0.0 (77.4)	1.8 ± 0.0 (82.0)	5.4 ± 0.0 (78.9)	0.53 ± 0.12 (87.2)
Japanese littleneck	4.3 ± 0.6 (80.0)	3.0 ± 0.0 (77.3)	3.2 ± 0.7 (81.4)	1.2 ± 0.1 (83.6)
Washington clam	no lipid data (77.0)	2.6 ± 0.4 (80.2)	5.2 ± 0.7 (78.0)	1.8 ± 0.0 (80.1)

total PCB levels in green mussel: Hong Kong (1986), 49.0-330 ng/g wet weight; south India (1988-1989), 0.66-7.10; coastal water of Thailand (1994 and 1995), 0.17-12.0 and <0.01-20.0, respectively; coastal water of India (1994-1995), 0.31-15.0; and coastal water of Philippines (1994-1997), 0.69-36.0. By comparing other countries, total PCB levels in mussel in this study were comparable or lower than those obtained from Europe and America, but comparable or higher than those obtained from India, Thailand and Philippines. This suggests that PCB sources may be less prevalent in Korea than Europe and America, but more prevalent than south Asian countries.

Total PCB levels of each shellfish are illustrated in *Fig.* 2. For clam-1, granular ark, and clam-2, decreasing trend from Mar. to Sept. 2002 was apparent, and the lowest in Dec. 2001. It was common that the lowest level was appeared in Dec. 2001 followed by increasing in Mar. 2002. The extraordinarily high total PCBs in Sept. 2002 characterized the distribution pattern of mussel. For Japanese littleneck and Washington clam, total PCB levels were diminished from June to Dec. 2002 and the level in Mar. 2002 was very close to that in Dec. 2001.

The extent of the correlation between the total PCB levels and the lipid content was relatively low, $r^2 = 0.65$. The PCB levels in marine organisms are also influenced by some other aspects, such as delivery of pollutants, physiological variation of an organism, and variation of the environmental parameters of ambient water.[22] It was reported that the age and sex of the mussel and the temperature and salinity of water have no significant effect on the accumulation of organochlorines.[23] Thus, the observed variation in PCB levels suggests the presence of local contamination sources.

4. Conclusion

We determined PCBs in tissue samples of seven species shellfish collected from a market, originated from the southern coastal areas of Korea. Although the production and practical use of PCBs was banned more than 20 years ago, they were still contained in shellfish tissues. The total PCB levels ranged from 0.5 ng/g d.w. (clam-2, Dec. 2001) to 34.5 ng/g d.w. (mussel, Sept. 2002). The four species, clam-1, granular ark, mussel, and oyster, contained relatively higher levels. The order of average PCB levels was as follows: mussel (13.0 ng/g d.w.) >> granular ark (8.3 ng/g)~clam-1 (8.0 ng/g) > oyster (6.0 ng/g) >> clam-2 (2.6 ng/g) > Japanese littleneck (1.6 ng/g)~Washington clam (1.3 ng/g). The total PCB levels in shellfish were much lower than reported levels in mussels from Central and South America and north-west Mediterranean coastal zone, but comparable to those from Singapore's coastline.

The most apparent variation in PCB level was the lowest in Dec. 2001 and a dramatic increase in Mar. 2002 and a decreasing trend from Mar. to Sept. 2002 for clam-1, granular ark, and clam-2. It was also common that the lowest level appeared in Dec. 2001. The extraordinarily high level in Sept. 2002 characterized the distribution pattern of mussel.

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